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Morphological Studies on the Hepatotoxic Effects of Various Inhalation Anesthetic Drugs

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Introduction

The liver is the largest; its functions are numerous and diversified; and experimentally, investigation is more difficult than of any other organ in the body.

Since GUTHRIE[1] reported the article entitled, "On Some Fatal After-effect of Chloroform on Children," in 1894, issue of the Lancet; a number of investigations into the effects of anesthetics upon the liver have been reported over the past seventy-five years. Recently, halothane (in 1956)[2,3] and methoxyflurane (in 1960)[4] the new halogenated hydrocarbons, were introduced to the practice of anesthesia.

Since 1958, several reports have appeared in the literature in which halothane was suspected of causing massive and fatal liver necrosis[5-14]. Within the past decade, the interest in the hepatic effect of various anesthetic agents, especially this new halogenated anesthetic, has increased and a great public concern has been directed to the hepatotoxicity of new halogenated anesthetics.

In 1966, the NAS-NRC (the Committee on Anesthesia of the National Academy of Sciences National Research Council) Subcommittee on the National Halothane Study[15] concluded that fatal postoperative massive hepatic necrosis was a rare occurrence and halothane had a record of safety compared to all anesthetics. However, the possible rare occurrence of halothane-induced hepatic necrosis following single or multiple administration could not be rule out and the study failed to establish a causal relationship between halothane and hepatic necrosis.

On the other hand, electron microscopy raised its maximal magnificaton from 1,000 folds to 150,000 folds or more, making us possible to observe the finer structure as well as the metabolic process being done in the liver cell. However, only few reports concerning the ultrastructural changes of the hepatic cell caused by anesthetic agents have ever been seen[16,17].

Therefore, this study has been made to compare possible hepatic changes subsequent to repeated anesthesia of various anesthetic agents by means of light and electron microscopy.

Materials and Methods

Adult white mice and albino rabbits were subjected to this study. Diethyl ether, cyclopropane, halothane, methoxyflurane (Penthrane) and chloroform were used as the causative agents. In order to emphasize the effects of these agents upon the liver cell, repeated administration was employed.

1) Mice :

One hundred sixty mice of the D. D. strain weighing 18 to 23 grams of both sexes were used. Because it might be assumed that the mouse liver responded to drugs in a manner similar to the liver of the human[18], mouse liver was chosen to preliminary study.

All animals were in good condition and were acclimatized at least one week prior to the study and then divided into eight groups of twenty mice each.

They were exposed to various anesthetic agents, to hypoxic and to hypercarbic atomospheres for one hour a day on seven consecutive days, except for control group having lived under standard conditions without exposure. (Table 1)

The experimental animals were put in a glass jar of approximately eight liter capacity and the jar was sealed with a vinyl cloth penetrated with two rubber tubes. Each anesthetic gases were delivered through one tube mixed with oxygen with high flow. Furthermore, to investigate the true effect of hypoxia or hypercarbia upon

TABLE 1

Experimental animals e. g. mice and rabbits were repeatedly exposed to following anesthetic agents, to hypoxic and hypercarbic atomospheres for one hour a day on seven consecutive days.

Group 1 ..... (control)
Group 2 ..... 4 % diethyl ether
Group 3 ..... 18 % cyclopropane
Group 4 ..... 1 % halothane
Group 5 ..... 0.5 % methoxyflurane
Group 6 ..... 0.7 % chloroform
. . . . . .
Group 7 ..... hypoxia (5% O_2 95% N_2)
Group 8 ..... hypercarbia (15% CO_2 : 85% O_2)

TABLE 2 Materials and Methods

	MICE	RABBITS
RESPIRATION	spontaneous	assisted
METHODS	repeated exposure (1 hour/day, 7 days)	
OBSERVATIONS	1) immediately after 7th exposure 2) 7 days after last exposure	1) before 1st exposure 2) after 3rd exposure 3) after 7th exposure 4) 7 days after last exposure
LIVER FUNCTION TEST		SGOT SGPT
SPECIMEN	taken after decapitation	taken by biopsy

the liver cell, in hypoxic group 5% oxygen was delivered with nitrogen and in hypercarbic group 15% carbon dioxide with oxygen. Diethyl ether and chloroform were vaporized in a copper kettle, halothane in a Fluotec and methoxyflurane in a Pentec.

Animals were sacrificed by decapitation on the last day of the serial exposure and on seven days after completion of the serial exposure respectively. (Table 2)

2) Rabbits:

Forty albino rabbits weighing 2000 to 2500 grams were divided into eight group as described under the experiment with mice. Each five animals of seven experimental group, without premedication, were exposed to various condition as illustrated in Table 1 by means of an ACOMA infant circle with a carbon dioxide absorbent for one hour daily on seven consecutive days. A special rabbit face mask was used for induction and maintenance, and to avoid any hypoxia or hypercarbia, the respiration was assisted manually throughout during anesthesia.

Liver biopsies and serum transaminase measurement (SGOT and SGPT) were done on each animals, 1. before the first exposure, 2. on the third anesthesia day, 3. on the seventh (last) anesthesia day, and 4. seven days after the last exposure respectively.

In control group, only liver biopsies and serum transaminase measurement were performed under local anesthesia at same intervals. (Table 2)

For electron microscopy, the liver was rapidly cut into tiny blocks (1mm in greatest dimension), which were fixed for 90 minutes at 4°C in 1% solution of osmium tetroxide adjusted with phosphate buffer to pH 7.4 and to which sucrose had been added. After dehydration in a graded series of ethyl alcohol, the tissue blocks were embedded in Epon 812[19]. Sections were cut on a Nippon-Denshi JUM-5A or Porter-Blum microtome with glass knives and then stained with lead citrate[20]. They were examined and photographed in a Hitachi HU-11A model microscope.

For light microscopy, specimens were prepared for hematoxylin-eosin, Sudan III and P. A. S. stain respectively.

Results

All the result are briefly summarized in Table-3 (mice) and in Table-4 (rabbits).

Group 1. Control (Normal hepatic structure)

It is found that the liver biopsy itself does not alter the structure and the function of the rabbit liver. A normal view of electron microscopy is presented in Fig. 1 (mouse) and in Fig. 2 (rabbit).

Two neighboring cells are in close contact, with a narrow intercellular space, 100-150 angstroms in width, extending between cell membranes. The bile canaliculus contains regular microvilli projecting into its lumen. It is separated from the narrow space between neighboring cells by fusion and condensation of the cell membranes.

The surface of the liver cell directed toward the Disse spaces has many microvilli. These increase the cell's surface and indicate active resorption of fluid. The Disse space is contact with the thin sinusoidal-endothelial cell. The continuous basement

TABLE 3

Results of light and electron microscopic examination of mice liver exposed for one hour daily on seven consecutive days.

		total number examined	normal	fatty infilt.	cellular change	endoplasmic reticulum	mitochondria	other findings
Killed immediately after the last exposure	Ether	10	9	1	0	sER increased	normal	glycogen decreased
	Cyclopropane	10	10	0	0	sER increased	normal	
	Halothane	10	3	7	0	degranulated	almost normal some odd shaped	liposome increased
	Methoxyflurane	10	0	9	1	degranulated dilated vesicularly	swollen	liposome markedly increased
	Chloroform	9	2	2	5	degranulated dilated irregularly	swollen crenated cristae short	liposome & free ribosome increased
	Hypoxia	10	9	1	0	normal	swollen crenated destructed	
	Hypercarbia	10	9	0	1	normal	slightly swollen and crenated	amorphous light area increased
Killed seven days after the last exposure	Ether	10	10	0	0	normal	normal	
	Cyclopropane	9	9	0	0	normal	normal	
	Halothane	10	10	0	0	normal	normal	
	Methoxyflurane	10	7	3	0	normal	some swollen odd shaped	a few giant liposome
	Chloroform	8	6	1	1	dilated partly	some swollen and crenated	
	Hypoxia	10	10	0	0	normal	almost normal some crenated	
	Hypercarbia	10	10	0	0	normal	normal	

TABLE 4

Results of micrographs and serum transaminase measurement of rabbits exposed for one hour a day on seven consecutive days.

	Before the exposure	On the 3rd day of exposure	On the 7th day of exposure	Seven days after the last exposure
ETHER	S. T. (30, 37)	S. T. (36, 43) normal (sER increased)	S. T. (43, 40) normal (sER increased)	S. T. (29, 35) normal
CYCLOPROPANE	S. T. (36, 40)	S. T. (53, 60) normal (sER increased)	S. T. (58, 62) normal	S. T. (35, 44) normal
HALOTHANE	S. T. (34, 30)	S. T. (37, 38) fatty infilt. (moderate) Mitochondria : some swollen. ER : slightly dilated, degranulated.	S. T. (38, 27) fatty infilt. (−) Mitochondria : partly swollen. ER : normal.	S. T. (37, 35) normal
METHOXYFLURANE	S. T. (33, 50)	S. T. (163, 76) ↑ fatty infilt. (moderate) Mitochondria : swollen. ER : dilated, degranulated. (markedly)	S. T. (55, 49) fatty infilt. (diminished) Mitochondria : swollen. ER : dilated, degranulated.	S. T. (52, 57) fatty infilt. (−) Mitochondria : almost normal ER : normal
CHLOROFORM	S. T. (28, 41)	S. T. (540, 1200) ↑ ↑ central necrosis fatty infilt. (moderate to severe) Mitochondria : swollen, crenated. ER : dilated→irregular vacuoles.	S. T. (46, 144) ↑ necrosis (more minimal) fatty infilt. (diminished) Mitochondria : swollen, crenated. ER : irregularly dilated.	S. T. (30, 43) necrosis (−) fatty infilt. (−) Mitochondia : partly swollen. ER almost normal
HYPOXIA	S. T. (29, 34)	S. T. (39, 76) Mitochondria · swollen, crenated. ER partly dilated.	S. T. (40, 63) Mitochondria : crenated. ER : normal.	S. T. (31, 37) normal
HYPERCARBIA	S. T. (40, 38)	S. T. (40, 43) normal	S. T. (32, 35) almost normal (some mitochondria crenated)	S. T. (19, 21) normal

S. T. ( ) : Serum Transaminase (SGOT, SGPT)
ER endoplasmic reticulum
fatty infilt. : fatty infiltration

Explanation of Figures

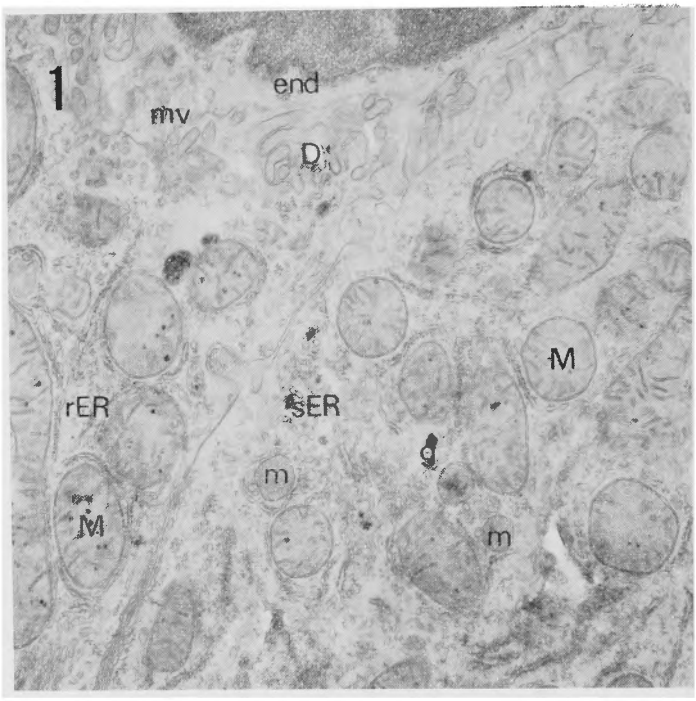

Fig. 1 Electronmicrograph of control mouse. × 15,000

Abbreviations

M : mitochondrion
m : microbody
rER : rough surfaced endoplasmic reticulum
sER smooth surfaced endoplasmic reticulum
G Golgi complex
g . glycogen
N : nucleus
mv : microvilli
D : Disse space
bc : bile canaliculus
end : endothelial cell
S : sinusoid
F : fat droplet

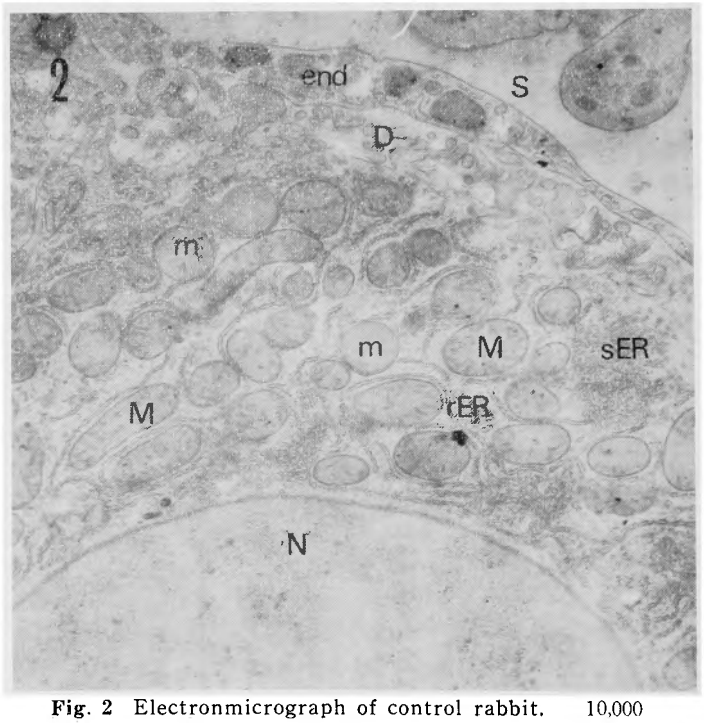

Fig. 2 Electronmicrograph of control rabbit. 10,000

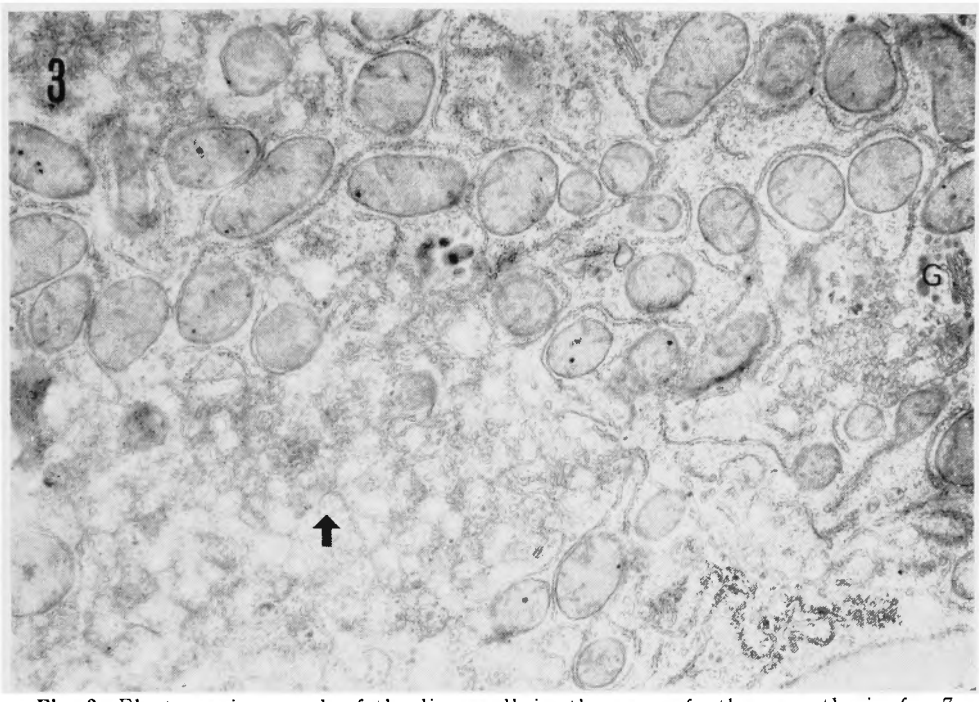

Fig. 3 Electronmicrograph of the liver cell in the case of ether anesthesia for 7 days. Normal size and shape of mitochondria, and normal profiles of endoplasmic reticulum. However, sER showes slight increase in number (↑). × 15,000 (rabbit)

Fig. 4 Portion of a cell from rabbit after 7 days of cyclopropane anesthesia. The organelles such as mitochondria and endoplasmic reticulum appear almost normal despite a small increase in the number of smooth vesicles of ER(↑). Glycogen granules are failed to stain so that they are seen as 'light holes' in cytoplasm. × 16,000

Fig. 5 Optical micrograph from mouse of halothane anesthesia for 7 days (Sudan III stain). Marked central fatty infiltration are shown.

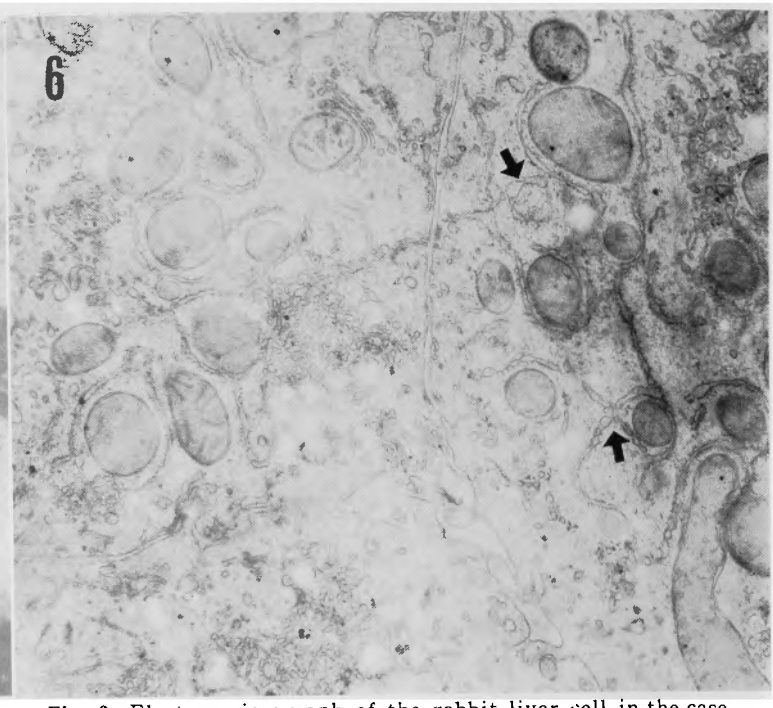

Fig. 6 Electronmicrograph of the rabbit liver cell in the case of halothane anesthesia for 3 days. The rER is slightly dilated, shattered and transformed into vesicle (↑). Some ribosome granules disappear. ⁄ 15,000

Fig. 7 Mouse liver of halothane anesthesia for 7 days. A few mitochondria are of odd shape, but remainders are within normal limits. Large fat droplets are seen in cytoplasm. Electron opaque bodies in Golgi complex are also shown(↑). ⁄ 10,000

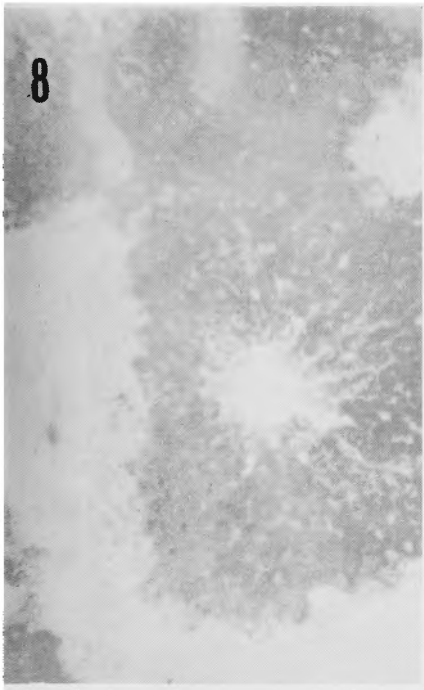

Fig. 8 Optical micrograph from mouse of methoxyflurane anesthesia for 7 days showes marked fatty infiltration by Sudan III stain.

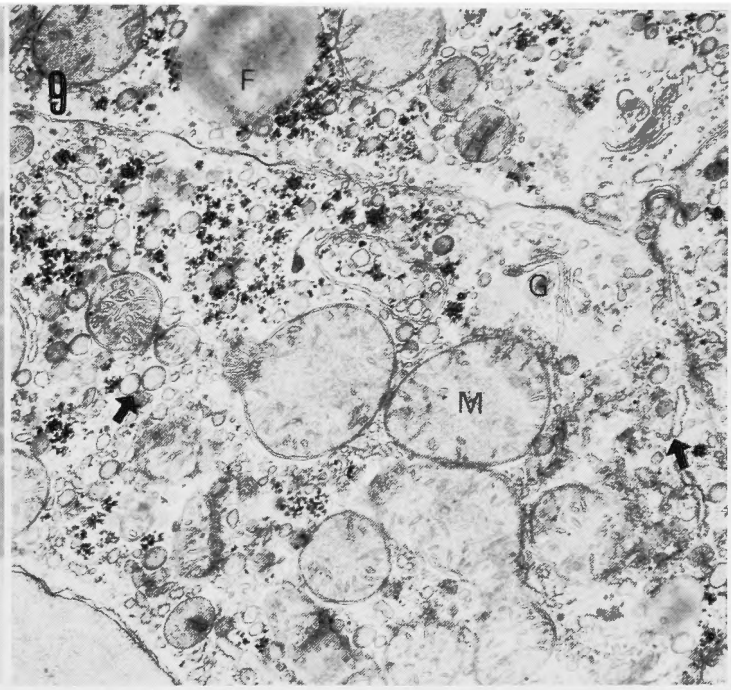

Fig. 9 Liver of methoxyflurane-anesthetized mouse for 7 days. Numerous small electron opaque lipid bodies and fat droplets are scattered throughout the cytoplasm(↑). Lipid bodies appear to be attached to the rER and in cisternae of Golgi complex. Mitochondria are swollen. × 17,000

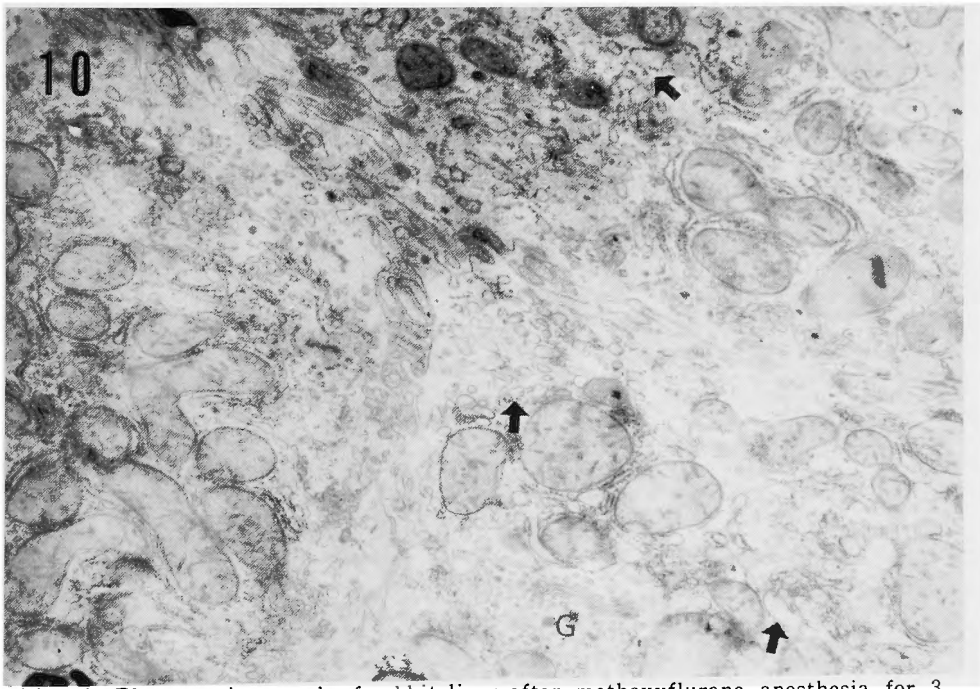

Fig. 10 Electronmicrograph of rabbit liver after methoxyflurane anesthesia for 3 days. Vacuolization of the cisternae of rER, and degranulation of its membrane (↑) are markedly evident when compared to halothane group. Some mitochondria are of odd shape. × 15,000

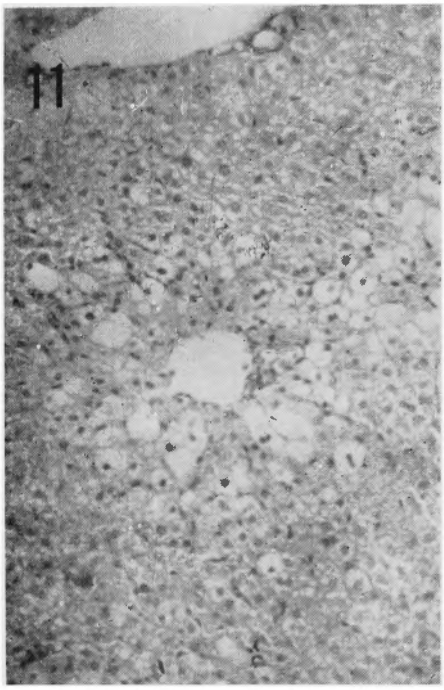

Fig. 11 Light micrograph of mouse liver cell. (chloroform). Central vacuolization and necrosis are seen. Fatty infiltration is not so prominent.

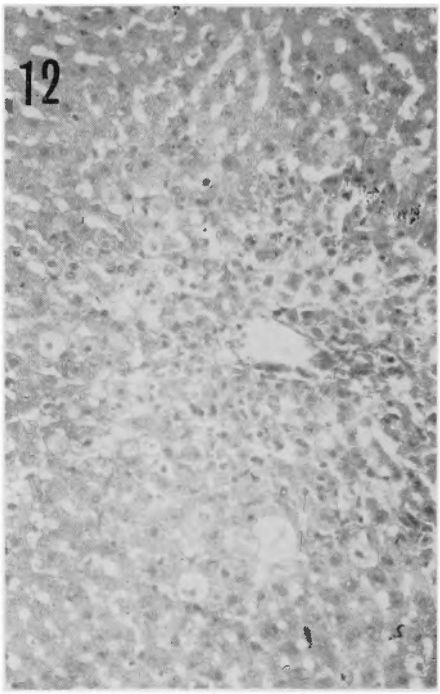

Fig. 12 Light micrograph of rabbit liver on the 3rd day of chloroform anesthesia. Central coagulation necrosis surrounded by moderate degree of vacuolization are shown.

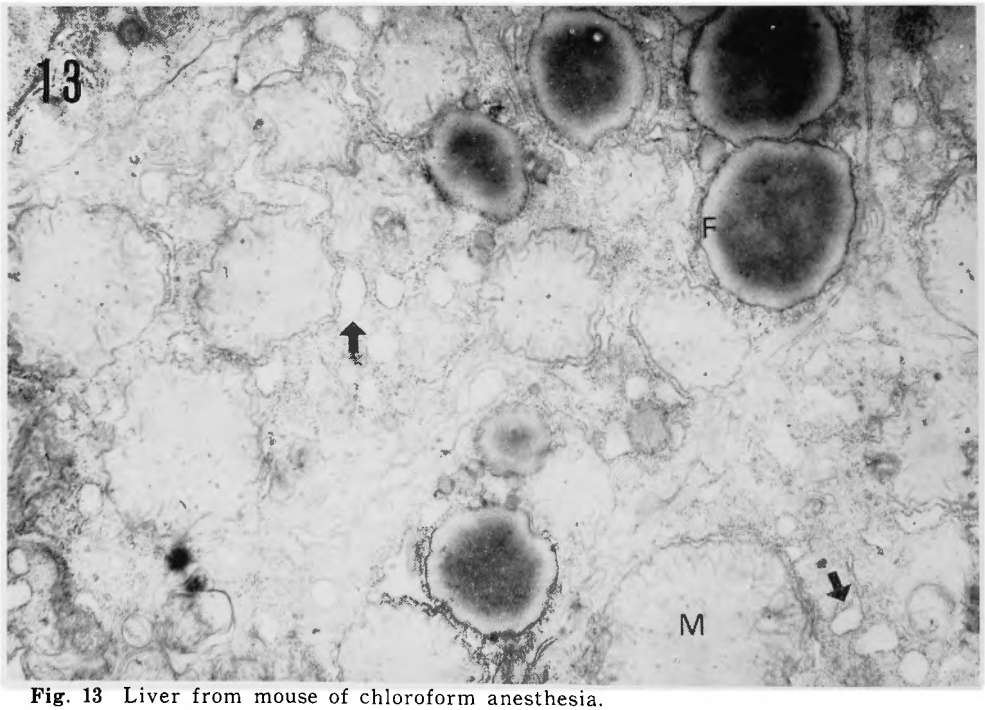

Fig. 13 Liver from mouse of chloroform anesthesia.
Mitochondria with marked crenation of mitochondrial membrane are swollen. Endoplasmic reticulum is irregularly and strikingly dilated (↑). Large fat droplets are noted. × 15,000

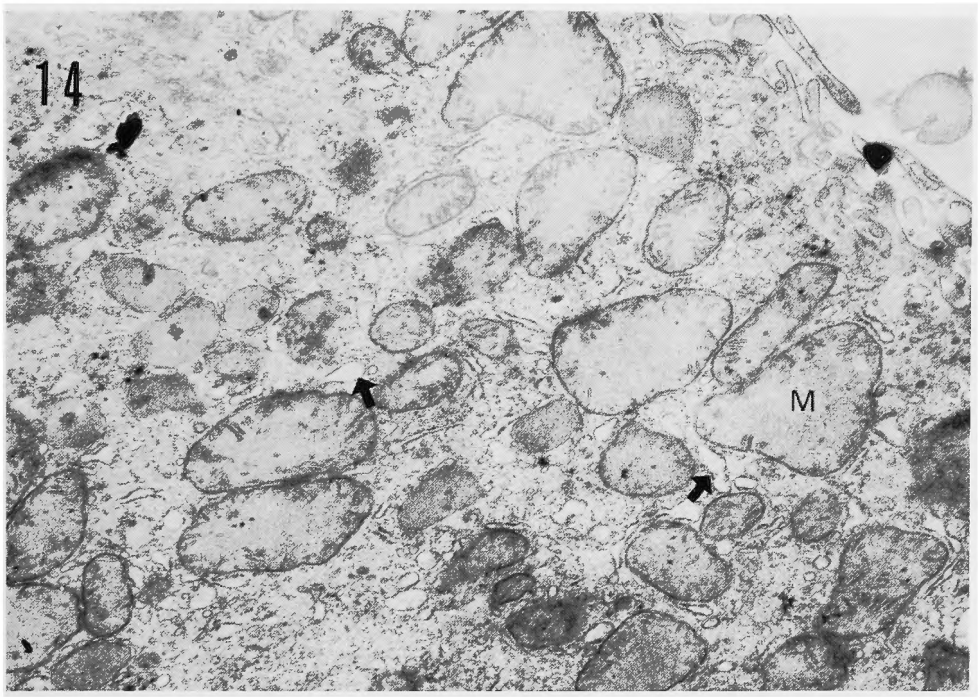

Fig. 14 Liver on the 3rd day of chloroform anesthesia (rabbit).
The picture demonstrates irregularly dilated rER (↑). Mitochondria are markedly swollen and crenated. × 15,000

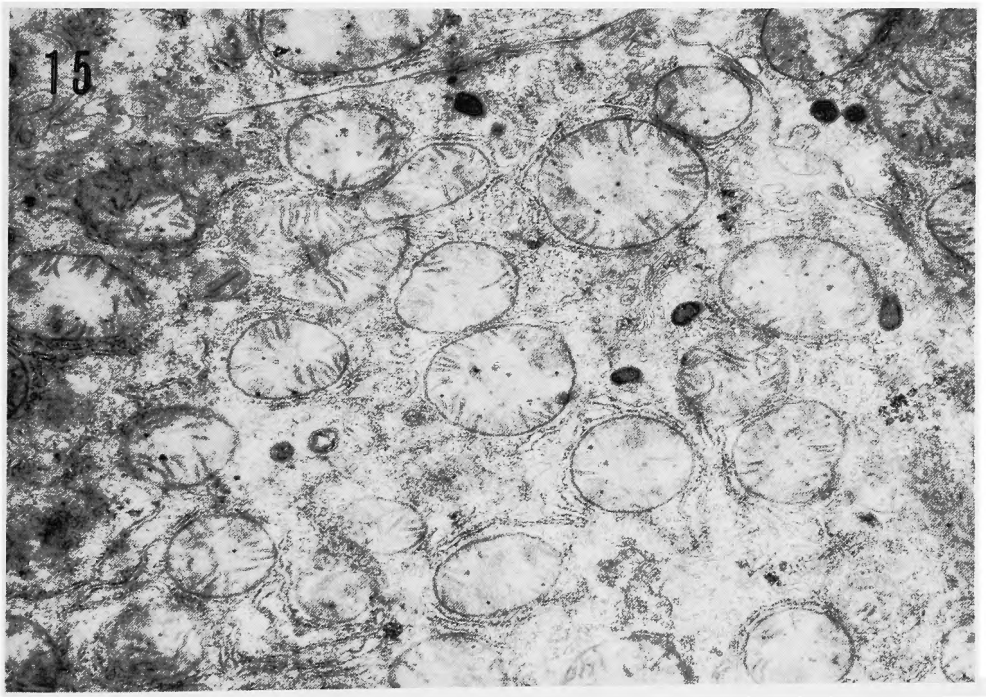

Fig. 15 Electronmicrograph of mouse liver in the case of chloroform anesthesia after the rest period of 7 days.
The organelles in this hepatocyte appear almost normal except the larger size of some mitochondria. × 15,000

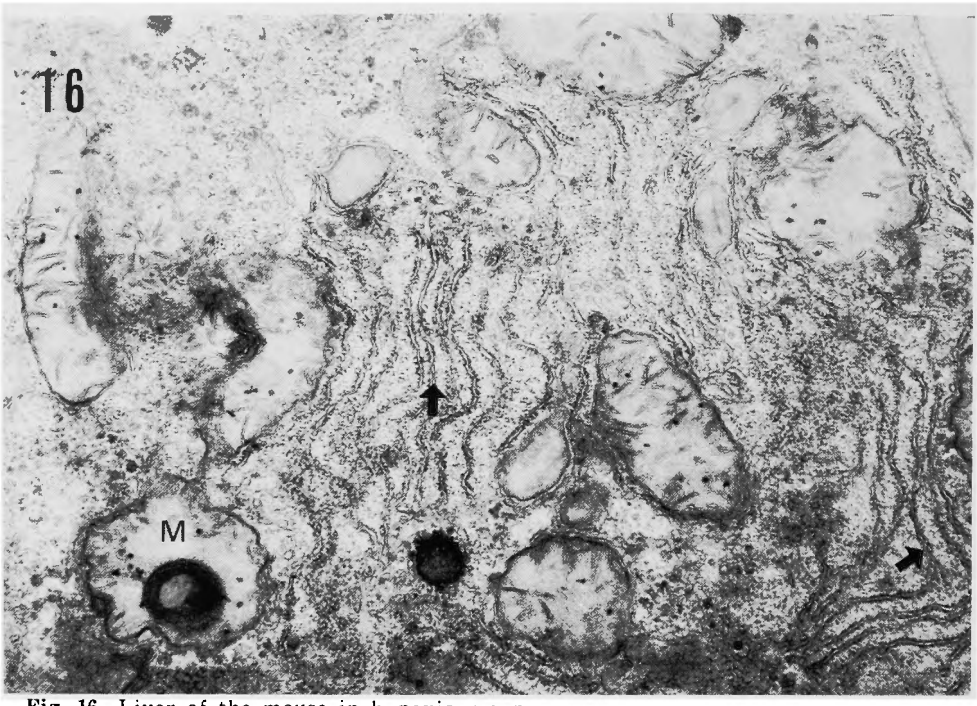

Fig. 16 Liver of the mouse in hypoxic group.
The crenation of mitochondrial membrane is noted. Mitochondria in the left are losing their own mitochondrial membrane. Mitochondrion in the lower left contains a myelin figure. However, endoplasmic reticulum appears almost normal (↑). × 18,500

Fig. 17 Liver of the mouse in hypercarbic group.
Amorphous light areas are abundant and some osmiophilic glycogen granules are observed here (↑). Mitochondrial membrane are somewhat crenated. × 18,500

membrane, which is characteristic of all other capillaries, is missing. This enhances the exchange between liver cell and blood, and justifies the distinguishing term "sinusoid".

The cytoplasm of the hepatic cells contains a large number of organelles: mitochondria, microbodies, lysosomes, endoplasmic reticulum (rough surfaced and smooth surfaced), and the Golgi complex. The cytoplasm also contains several types of inclusions: glycogen, fat droplet and pigments.

The mitochondria are composed of electron-dense matrix surrounded by a double membrane. The inner one invaginates into a central matrix as cristae mitochondriales, usually tubular in form. Within the matrix are the osmiophil mitochondria granules whose properties are unknown. Mitochondria vary in number, size, shape and structure during the various functional stages and represent about 25 per cent of the cell mass. Mitochondria are the main carriers of enzymes in the liver cell; several oxidative enzymes such as those of the citric acid cycle (Krebs' cycle) are present here. Adenosine triphosphate is synthesized in the mitochondria.

Microbodies are oval organelles surrounded by a single membrane, whose center is frequently occupied by either a dense or a lamellar nucleus. They are numerous in regenerating liver. Some believe they are mitochondrial precursors.

The endoplasmic reticulum represents to some degree a direct continuation of the folds of the cell membrane which may extend to the interspace of nuclear double membrane. This system undergoes continuous change and has to be considered as a transport path within the cell. The connection of membranes of the endoplasmic reticulum with small granules 150 Å in size, the ribosomes, is described as rough surfaced endoplasmic reticulum; its main function is intracellular protein synthesis. The vesicular and agranular form of endoplasmic reticulum is represented as smooth surfaced endoplasmic reticulum and is closely related to glycogen accumulations in the cell and must therefore play a part in the glycogenesis and the glycolysis.

The Golgi complex may appear in multiple from in the region between nucleus and bile capillaries, close to the latter. The vesicles contain droplets of fat or are empty.

Glycogen is evenly distributed over the cell. The glycogen particles are without distinct boundary and approximately 150–350 Å in size.

Fat droplets are extremely osmiophil. Owing to their compactness, larger fat drops exibit frequently wavy lines (shatter) due to sectioning technique.

Group 2. Diethyl Ether

Under light microscopy, no significant changes are demonstrable in the liver of mice and rabbits during or after repeated ether anesthesia.

When viewed in the electron microscope (Fig. 3), almost normal configuration of endoplasmic reticulum both rough and smooth, can be observed. Mitochondria are well preserved. An increase in smooth surfaced endoplasmic reticulum and a decrease in glycogen areas are observed when compared to normal liver, however, glycogen areas reappeared essentially the same as in control liver seven days after the last exposure.

Group 3. Cyclopropane

Both under light and electron microscopy, no trace of reactive or degenerative changes is detectable anywhere in hepatic cells of mice and rabbits, except for a slight evidence of a decrease in the amount of glycogen granules. These findings are the same as ether group described above. Furthermore, careful examination microscopically fails to demonstrate any significant differences between the two groups (Fig. 4).

Group 4. Halothane

Under light microscopy seven out of ten mice in this group are shown marked central fatty infiltration but no course of cytoplasmic vacuolization and necrosis are recognizable (Fig. 5). Also in rabbit cases, moderate fatty infiltration is observed in the areas of central vein on the third day of serial exposure. But from Table 3 and 4, it will be noted that evidence of fatty infiltration is no longer discernible seven days after completion of the serial exposure. Fatty changes produced by halothane in mouse and rabbit liver are usual transient type.

Electron microscope reveals conspicuous change in relation to fatty infiltration and the endoplasmic reticulum. The rough endoplasmic reticulum is slightly dilated, shattered and transformed into vesicle. Some ribosome granules disappear (Fig. 6).

On the other hand, numerous small lipid bodies, 300-2400 Å in diameter, are scattered throughout the parenchymal cell cytoplasm. Usually these lipid bodies appear electron-opaque, but if the fixation is incomplete or not enough, lipid bodies exhibit only a peripheral rim of dense material surrounding a clear core.

Each small lipid bodies are always enclosed within a vesicle. Often these vesicles may be observed in continuity with the dilated endoplasmic reticulum or in association with the Golgi complex (Fig. 7). Small lipid bodies are fusing with one another and sometimes seen apparently attached to fat droplet. Small lipid bodies, however, are very rarely seen in normal liver, and only a few scattered fat droplets are present.

Mitochondria enlarge slightly, however this change does not involve mitochondria of all cells equally. Mitochondrial membrane and cristae are well maintained. Microbodies, mitochondrial precursor, appear to increase in number. But no striking alterations are observed in other cytoplasmic organelles of liver parenchymal cells.

In rabbits these findings are most prominent on the third day of anesthesia. But on the seventh day of anesthesia, these changes, particularly in the endoplasmic reticulum, tend to return normal. Seven days after the last exposure, the fine structure of hepatic cells appears essentially the same as in control liver.

Group 5. Methoxyflurane

From Table 3, it will be noted that a high percentage of livers examined immediately after seven exposures in mice show evidence of marked fatty infiltration by Sudan III stain, but seven days later it is seen only in three out of ten mice (Fig. 8).

In rabbits, also, the fatty changes are markedly observed on the third day of anesthesia, but tend to be diminished on the seventh day of anesthesia, and are no longer found in any specimen seven days after completion of the serial exposure. Therefore, these fatty changes produced by methoxyflurane are also the usual transient

type as observed in halothane group.

Electron microscopical alterations in the morphology of the granular endoplasmic reticulum are similar to those of halothane anesthesia described above, but the degree is somewhat more severe than in halothane and less than in chloroform. These changes associated with vacuolization of the cisternae of the rough endoplasmic reticulum, degranulation of its membrane, and the appearance of increased number of small lipid bodies are markedly evident in methoxyflurane group when compared to halothane group (Fig. 9 and 10).

On the other hand, alterations of mitochondria in this group are noticeable and characteristic when compared to halothane group; many of the mitochondria are swollen or enlarged, and partly devoid of limiting membrane. However, crenation of mitochondrial membrane is not observed. This is in contrast with the enlarged mitochondria appearing in some of chloroform group (Fig. 13) and hypoxic group (Fig. 16), where the limiting membrane is markedly crenated. The density of mitochondrial matrix is almost normal, or somewhat less electron-opaque than control group.

Seven days after the cessation of repeated methoxyflurane anesthesia, small lipid bodies (liposome) virtually disappear from the endoplasmic reticulum, while only a few giant liposomes are present. Although some of the mitochondria are still enlarged and odd shaped in some cells, the hepatocytes generally seem to return to normal.

Group 6. Chloroform

Two out-of twenty mice died during seven exposures of 0.7% chloroform and one died during rest period. At necropsy, severe centrolobular and midzonal necrosis which extends in many areas is present in the histologic sections.

In light micrographs, five of nine mice sacrificed immediately after the last exposure show cytoplasmic vacuolization about the central lobular portion and some of these produce central necrosis (Fig 11). Fatty infiltration is not so prominent as in methoxyflurane anesthesia in mice.

In rabbits on the third day of chloroform anesthesia, microscopic examination reveals central acidophilic coagulation necrosis surrounded by moderate to severe degree of fatty change in all animals in this group (Fig. 12). In some of them the sinusoidal spaces are dilated and congested with red cells and in some area frank hemorrhage is present.

On the last day of serial exposure, there is tendency to slight recovery in hepatic cells even though repeated exposures have been succeeded by this time; that is, portions of necrotized areas are smaller and midzonal fatty infiltration is more minimal than on the third day.

Seven days after completion of the serial exposure, necrosis, vacuolization and fatty infiltration are almost faded away from liver of mice and rabbits in light microscopy.

Under electron microscopic observation, the structure of the endoplasmic reticulum, mitochondria and Golgi apparatus of the liver of both experimental animals is strikingly altered. Enlarged and ballooned mitochondria with irregular cristae

locating in the periphery of the organella are seen and mitochondrial membrane is crenated and bizarre in shape (Fig. 13). The mitochondrial matrix may be more electron-opaque than other experimental groups.

The endoplasmic reticulum also undergoes various changes; the profiles dilate and are transformed into irregular vacuoles (Fig 14). Further more, some of Golgi complex appear to form lamellar whorls.

The ribosomes lose contact with endoplasmic reticulum rough surfaced, with concomitant increase in the number of free ribosomes. Small fragments of normal rough endoplasmic reticulum persist only around the mitochondria.

Small lipid bodies and large fat droplets also appear in association with the endoplasmic reticulum as seen in halothane and in methoxyflurane. Espcially, large fat droplets are more abundant in this case.

At the end of seven days after the last exposure, the mitochondria are less large and less bizarre in shape. The number of irregular cristae are reduced The endoplasmic reticulum appears almost normal except for persistence of the pericanalicular autophagic vacuoles and the large Golgi apparatus previously noted. Although large fat droplets and glycogen depletion are still found in some cells, these are not uniform and changes in any single cell are not so severe as during exposures (Fig. 15).

Group 7. Hypoxia

No impotant changes are observed light-microscopically, besides only one mouse produces moderate fatty infiltration.

The most striking cytological changes revealed by the electron microscope are crenation of the enlarged mitochondria and an increase in the number of cristae mitochondriales. Some mitochondrial membrane are becoming disintegrated partly (Fig. 16).

Other membrane structures, i. e., the endoplasmic reticulum, Golgi apparatus and cell membrane, seem unchanged in mice liver. However, in some rabbits livers slightly dilated profils of endoplasmic reticulum are seen on the third day of exposure.

Group 8. Hypercarbia

Except for one mouse, where there appear to be a pathologic vacuolization, the body and nucleus of the parenchymal cells show no changes indicative of serious damage.

In electron micrographs, an amorphous light area, suspected glycogen area, is more abundant than in other groups and it persists seven days after the last exposure (Fig 17).

Some osmiophilic granules are observed here and there in this amorphous light area whose relationship to the glycogen area is at present unknown. Some mitochondria are crenated in slight degree. The remainder of the cell structure appears almost normal.

Summary of the serum transaminase measurement

The significance of change in the serum transaminase activity has been considered as reflecting injury or disease of hepatic cellular element. In the present study, this estimation is used to observe the correlation of both function and morphology.

Table 4 shows serum glutamic-oxaloacetic (SGOT) and glutamic-pyruvic (SGPT) transaminase of rabbit case determined prior to exposure, on the third day of exposure, on the seventh (last) day of exposure, and seven days after the last exposure respectively. In rabbits normal SGOT and SGPT levels are ranged from 15 to 60. There appear to be a close relationship between the serum transaminase readings and the findings of light microscopy.

In diethyl ether, cyclopropane, halothane, hypoxia and hypercarbia groups, there is no increase in the serum transaminase readings on the whole course of this experiment.

Methoxyflurane shows a slight increase in the serum transaminase on the third anesthesia day.

In chloroform group, marked increase occurs on the third day of exposure when observed central coagulation necrosis, but while the microscopical alterations tend to be diminished the readings also return to within normal limits.

Discussion

Theoretically, almost all of anesthetic drugs are capable of being cytoplasmic depressant, albeit generally reversible poisons, but only the most potent drugs may sometimes produce profund morphological changes in a sufficient degree of severity[21].

Since GUTHRIE[1] first described as a liver death following chloroform anesthesia in the English literature, a number of reports have appeared testing the effects of various anesthetic agents upon the liver. But the results from these investigations are different in some degree. There are several reasons why differences of opinion have developed.

It is one reason that coexisting factors active in the organism may mislead one in the true interpretation of hepatotoxicity.

Clinical results are sometimes misleading, for usually a number of factors other than the anesthetic agents lead to the development of postoperative liver damage. The factors of the depth of anesthesia[22], duration of anesthesia[23], the site of operation[9,24], the coincident administration of a great many other drugs[25,26], hypotension[27], hypoxia[28], hypercarbia[29], nutritional status[30], blood transfusion[31], various infectious processes[32], and metabolic disturbance[33]. If even can these other hepatotoxic factors be excluded as much as possible, a controlled experiment in man designed to test the hepatotoxicity of the drugs is impossible.

In laboratory animals, these factors can be excluded in some degree, but the factors of species difference or variation of sensitivity reaction must always be born in mind in the interpolation to man[34].

This study was undertaken to elucidate whether the various anesthetic agents commonly used might reveal any latent viscerotoxic properties to hepatic cells of laboratory animals e. g. mice and rabbits under electron microscopy not apparent under light microscopy.

In clinical anesthesia, it has been empirically known that diethyl ether and cyclopropane innocent to liver cell if the anesthesia does not exceed the clinical level.

In early investigations, it was rarely reported that significant histological damage which varied from slightly fatty infiltration to a frank central necrosis similar to that produced by chloroform could occur following the administration of diethyl ether[35,36] and cyclopropane[37] anesthesia; however, there was reason to doubt that ether and cyclopropane were true hepatotoxins but rather in such instances other factors were at play.

In this study, the ultrastructual investigation revealed that these two anesthetic agents caused no significant effect upon the hepatic cell organelles, but merely an increase in smooth surfaced endoplasmic reticulum and a decrease in glycogen areas were observed.

These findings in hepatic cell organelles were nonspecific quantitative alterations but not qualitative.

The smooth surfaced endoplasmic reticulum is localised quite close to the glycogenrich regions and is in close connection with glycogenesis and glycolysis[38,39]. During fasting, the smooth surfaced endoplasmic reticulum can be found around the few remaining areas with glycogen, while during glycogen storage the amount of smooth surfaced endoplasmic reticulum decreases, probably because it is used up in this process[39].

However, these observations concerning glycogen and smooth surfaced endoplasmic reticulum were not specific in ether or cyclopropane anesthesia but in all anesthetized groups were observed in various degrees.

Hyperglycemia with concurrent reduction in the glycogen content of the liver during anesthesia, particularly with ether, has long been recognized and its mechanism has been called adrenal-sympathetic activity[40]. But PHADAK[41] suggested the mechanisms might be somewhat different to consider that the smooth endoplasmic reticulum was the site of glucose-6-phosphates. There was also suggestive evidence that anesthesia might interfere with cellular transfer of glucose thereby impeding the phosphorylation and subsequent metabolism of glucose.

On the other hand, SMUCKLER[17] reported isolated perfused liver as the test material that the liver perfused with ether failed to show the structural change in the endoplasmic reticulum and alteration in glucose-6-phosphate activity.

There is continued concern as to whether hepatic dysfunction may result from the use of halogenated anesthetic agent. Since it was known that many halogenated hydrocarbons, such as chloroform and carbon tetrachloride, were hepatotoxic, there was obvious concern initially that halothane and methoxyflurane, which belonged in the same category of agents, might behave similarily. However, nowadays it is said this fear seems to be groundless.

Under electron microscopy the most common cellular response to the halogenated anesthetics; halothane, methoxyflurane and chloroform, were concerning with the degranulated or dilated endoplasmic reticulum, varying in degree from small vesicules in halothane to multiple irregular vacuoles in chloroform. These changes also accompanied with fatty infiltration under light microscope and an increasing number of small osmiophilic lipid bodies under electron microscope. However, these cytoplasmic bodies are by no means peculiar to repeated exposure to halogenated anesthetics;

similar structures have been observed in liver of normal fasted mice[42], in liver of mice fed choline-deficient diets[43] and in liver of rats after the administration of ethionine[44].

The exact composition of these osmiophilic bodies is not known, but it has been pointed out that, in osmium tetroxide-fixed material, both fat droplet and small cytoplasmic bodies are exteremely electron opaque. Because of these similarities between small cytoplasmic bodies and fat droplets, it is suggested that the small cytoplasmic bodies contain at least some component of lipid[45]. Other investigators have reached similar conclusions; NOVIKOFF et al[46] have presented histochemical evidence that similar osmiophilic bodies appearing after feeding a diet supplemented with orotic acid are lipid in nature.

BAGLIO et al.[47] have described that additional evidence suggesting their lipid nature is as follows: 1) the close association of these bodies with abnormal accumulation of triglyceride in the liver; 2) a close correlation between the time sequence of the increase in liver triglyceride level and the appearance of these bodies; and 3) the fact that the larger adielectronic bodies which have been recognized for years as lipid in nature, are obviously derived by fusion of the small osmiophilic bodies.

Although it has been postuated that these bodies are the probable precursors of the fat droplet, it remains unknown whether these small lipid bodies are entering the hepatic parenchymal cells or whether they are being synthesized within the cells.

REBOUÇAS et al. have described that the production of the fatty liver probably involves one or more of the following four processes[48]: 1) increaseed hepatic lipid synthesis, 2) decreased hepatic lipid utilization (e. g. oxidation), 3) increased transport of fatty acids from peripheral fat depots or 4) decreased release of lipid from the liver. They have also concluded that fatty liver is not directly related to an increased synthesis or a decreased oxidation of fatty acids in the liver. Derangements in lipid transport are probably the important factor in the production of the fatty liver.

CASLEY and SMITH[49] have called structures, which have the same size range and fixation properties as the bodies observed, lipoprotein. It has been also suggested that the endoplasmic reticulum may be concerned with protein synthesis and be considered as a transport path within the cell. Furthermore, the process by which lipoprotein are secreted from the liver into the circulation, via the hepatic sinusoid, is thought to involve the endoplasmic reticulum.

Alterations of the endoplasmic reticulum and appearance of small lipid bodies after repeated exposure of halogenated anesthetics suggest probably a block in the synthesis of lipoprotein by the liver or in the transfer of this plasma protein to the blood. Namely, it may be represented that the engorgement of the endoplasmic reticulum by the small lipid bodies is probably the earliest morphological expression of the interference in lipoprotein metabolism. Furthermore, the present experiment reveals the degrees of these alteration differ markedly; dilatation of the endoplasmic reticulum is regular and transient in the case of halothane and methoxyflurane compared to chloroform.

On the other hand, summary of the mitochondrial changes of the three halogenated anesthetics is as follows: 1) almost normal mitochondria in halothane group, 2) swollen mitochondria in methoxyflurane, and 3) enlarged and crenated alterations in chloroform. There are many differences among these anesthetics.

In 1954 CHRISTIE et al.[50] suggested in a study of the mechanism of action of hepatotoxin that the primary locus was the membrane of the liver mitochondria. DIANZANI[51] showed that after hepatotoxin, there was evidence for uncoupling of oxidative phosphorylation, loss of mitochondrial pyridine nucleotides, and lowering of the liver content of adenosine triphosphate.

However, RECKNAGEL et al.[52] have recently indicated that integrity of liver mitochondria is not seriously alterted in the early peroid after administration of carbon tetrachloride.

They have also shown that mitochondrial respiratory control is unimpaired, whereas enzymes of the endoplasmic reticulum are undergoing pathological changes and liver triglyceride content is increased. The conclusion is drawn from this study that the primary target for the attack by hepatotoxin is the hepatic endoplasmic reticulum, not mitochondria.

KLATSKIN[53] has suggested that in the case of exposure to hepatotoxin, the inital event appears to be a destructive peroxidation of the lipids in the membranes of the endoplasmic reticulum. This leads to impairment of protein synthesis and, secondarily, to alteration in mitochondrial permeability. As a result, there is a loss of DPN (diphosphopyridine nucleotide), a decrease in the activity of the DPN-linked dehydrogenases of the Krebs cycle, a decline in ATP and uncoupling of oxidative phosphorylation. This chain of events, leading inhibition of protein synthesis and energy production, ultimately result in cell death.

This study presented above does not provide the definitive answer as to the mechanism involved in the rare occurrence of holothane-induced hepatic necrosis following clinical anesthesia. It serves to emphasize, however, that in halothane and methoxyflurane, the new halogenated anesthetics, there is a latent depressant property of intrahepatic block in triglyceride transport secondary to inhibition of lipoprotein synthesis.

On the other hand, it is a clear-cut evidence that chloroform is more toxic than the other two halogenated anesthetics. The changes that observed with chloroform involve both mitochondria and the endoplasmic reticulum in severe degree. The mitochondrial changes, especially crenation, are similar that observed in hypoxic group, and are not readily reversible. Although there is a small but vocal opposition that insists that the dangers of chloroform have been magnified and distorted to an unreasonable degree[54,56], chloroform may have acted as a hepatotoxin similar in nature to carbon tetrachloride[56], true hepatotoxin.

Summary and Conclusion

A comparative study of the effects of diethyl ether, cyclopropane, halothane, methoxyflurane and chloroform on the hepatic cells of mice and rabbits in repeated

use was performed by means of light and electron microscopy.

The results obtained can be summarized as follows:

1. In the diethyl ether and cyclopropane groups, no significant alteration of the finer structures of mice and rabbits liver cell was demonstrated except for an increase in the amount of smooth surfaced endoplasmic reticulum and some decrease in the glycogen areas.
2. Both in halothane and methoxyflurane groups, similar changes were found on optical microscopic examination, i. e., a fatty infiltration in the cytoplasm, but no vacuolic or frank necrosis was seen.

   Electron microscopic study revealed that numerous small electron opaque lipid bodies and large fat droplets were scattered throughout the parenchymal cytoplasm and that the profiles of endoplasmic reticula were Dilated, shattered, and transformed. This view may suggest an early stage of the disturbance of lipoprotein metabolism in the liver cell. Mitochondria showed no significant deteroration, except that some of them were slightly swollen and the mitochondrial membrane was partially destructed in the methoxyflurane group.

   These changes were reversible and almost restored to normal after seven days.
3. In chloroform group, many experimental animals showed central vacuolization and central necrosis. Under electron microscopic observation, the structure of the endoplasmic reticulum, mitochondria and Golgi complex were strikingly altered. These changes were not readily reversible. The hepatotoxicity of chloroform in cell organelles was confirmed.

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和文抄録

各種吸入麻酔薬の肝毒性に関する形態学的研究

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麻酔薬の肝毒性に関する問題には，クロロホルムの肝障害以来，多くの関心が向けられてきた．特に最近，新しいハロゲン化麻酔薬であるハロセン，メトキシフルレインが広く臨床に使用され，かつ又その肝毒性が問題になっている折から，麻酔薬の肝臓に及ぼす影響が再び盛んに検討される様になった．

しかるに，この問題に関する今までの研究は，肝機能検査や光学顕微鏡を用いたものが多く，最近とみに解像力を増している電子顕微鏡を用いたものは極めて少い．

本研究は，現在主に用いられている麻酔薬の肝微細構造に及ぼす影響を，電子顕微鏡を中心として形態学的に再検討し，合せて現在問題になっているハロセンの肝毒性の原因についても考察した．

実験動物にはマウスと家兎を，麻酔薬にはエーテル，サイクロプロペイン，ハロセン，メトキシフルレイン，クロロホルムを用いた．

又麻酔薬の肝臓への作用を強調するため，各々臨床使用濃度で1日1時間ずつ7日間の繰返し投与を行った．尚この他，同様な方法で，低酸素及び炭酸ガス蓄積のみの影響をも比較検討した．

試料はマウスについては7日間の実験終了直後と，更に7日間の回復期間をおいたものとの二回に分け，いずれも断頭致死せしめて採取した．又家兎では実験前，実験第3日目，実験第7日目及び実験終了後7日目に各々biopsyにより採取し，同時に肝機能をも比較するため，血清トランスアミナーゼを測定した．

非ハロゲン化麻酔薬であるエーテル，サイクロプロペインは共に電子顕微鏡所見として，非特異的な滑面小胞体の量的な増加とグリコーゲン顆粒の減少を認めるのみで著明な変化はなかった．

新しいハロゲン化麻酔薬であるハロセン，メトキシフルレインについては，特に小胞体に程度の差はあるが共通点がみられた．即ち，粗面小胞体は一部脱顆粒を起して胞状に開大し，その中にsmall lipid bodyを含んでいる所見がみられ，それが融合して脂肪滴に変化していた．これらは又光学顕微鏡では脂肪沈着としてみられた．　脂肪滴のPrecursorであるsmall lipid bodyは，以上の如く小胞体と密接な関係を有するが，通常脂肪は蛋白合成の場である小胞体に於いてLipoproteinとなり，更に小胞体を通って血中に放出されると考えられている．

ハロセン，メトキシフルレインに見られる変化は，転送されるべき脂肪が小胞体内に蓄積したものとも考えられ，これらの麻酔薬が小胞体或はそれに含まれる酵素に何らかの抑制作用を有することが示唆される．又メトキシフルレインはハロセンに比し，小胞体の変化の程度がより大きく，又ミトコンドリアについても膨化等の軽度の変化を認めた．尚，これらの変化はいずれも7日間の回復期間後には消失し正常に復していた．

クロロホルムでは，約半数に中心性壊死が認められ，電子顕微鏡でもミトコンドリアの膨化，限界膜の鋸歯状凹凸，又クリスタの配列異常等の変性像がみられた．小胞体についても，ハロセン，メトキシフルレインよりも更に強度な開大と不正空胞化が認められた．血清トランスアミナーゼではクロロホルムのみ著明に上昇し，特に実験第3日目に最高となり，以後形態学的所見の改善と共に下降した．

以上各種吸入麻酔薬の繰返し投与の肝微細構造の変化を電子顕微鏡を中心として観察したが，(1)エーテル，サイクロプロペインは肝細胞には殆んど無毒であり，(2)ハロセン，メトキシフルレインは小胞体系の抑制による一時的な脂質の転送過程の障害を認め，(3)クロロホルムは各細胞小器官に多様性の変化を招来し，かなり強い肝毒性を示した．